Chapter 15

Stem Cells for Bone Regeneration: Role of Trophic Factors

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Abstract

Stem cells play a critical role in tissue regeneration and repair, maintenance and turnover and the control of haematopoiesis in the various tissues. These cells have an incredible ability to differentiate into specific cell types like osteoblasts, chondrocytes or myocytes and to develop bone, cartilage or muscle tissues. Now it is believed that the cells do not differentiate by themselves but rather the secretion of the bioactive (trophic) factors which are responsible for the functional outcome of the tissue. Stem cells reside in complicated and dynamic three-dimensional (3D) microenvironments in vivo known as stem cell niches. The niches are composed of extracellular matrix (ECM), soluble and tethered proteins and supporting cells, which have a profound influence on the functionality of the cells, including differentiation and trophic factor release. In this chapter, we review and emphasize the influence of stem cell microenvironment on the secretion of trophic factors and their perspective application for bone regeneration.

Keywords: bone, stem cells, tissue regeneration, niche, trophic factors

1. Introduction

Bone is a highly vascularized tissue with an intrinsic property to self-repair, regenerate and remodel. It has an excellent ability to heal traumatic injuries (e.g. fractures) without any formation of scars. However, there still exist a number of clinical scenarios where their self-repair and regenerative capabilities fail. Some of the classic examples include large bone defects caused by traumatic injury, infection, tumour resection and skeletal abnormalities due to congenital diseases. Bone-related injuries resulting from these clinical scenarios have

significant impacts on the health and lifestyle of individuals. In the USA alone, more than half a million patients experience problems due to bone defects each year, with medical cost associated with these defects being more than $2.5 billion/annum and this figure is expected to double by 2020. It is estimated that about 2.2 million bone graft procedures are performed around the world annually [1–3]. The current strategies used for augmenting bone regeneration include different bone-grafting methods, such as autologous bone grafts and allografts [4]. Autologous bone grafts have relatively successful clinical outcomes; however, donor site morbidity, limited supply and the complicated surgical procedures associated with bone harvests hinder the efficacy of such procedures. On the other hand, allogenic bone grafts are excellent in terms of sourcing large quantities of donor tissue required for treating large bone defects; however, the issues related to immunogenicity, rejection reaction and disease transmission render this treatment less ideal [4, 5]. The shortcomings associated with these treatments have led to exploring tissue engineering approaches and stem cell-based therapies for bone repair.

There is great promise for stem cell-based therapeutics for the treatment of numerous diseases and injuries; as such, substantial investment has been made over the past decade for new therapies. Stem cells play a critical role in tissue regeneration and repair, maintenance, turnover and the control of haematopoiesis in the bone marrow. They are considered as an attractive cell population for bone repair due to their proliferation, osteogenic potential and secretion of potent endogenous trophic factors to enhance local vascularization. These cells have an incredible ability to differentiate into specific cell types like osteoblasts, chondrocytes or myocytes and to develop bone, cartilage or muscle tissues. It is believed that the stem cells can help in repairing the damaged tissue not only by direct differentiation process but also indirectly through the secretion of their bioactive (trophic) factor [6]. In case of any tissue damage, the stem cells can be attracted to the damage site wherein they secrete bioactive factors that can function to trophically assist the repair and regeneration process.

In this chapter, we discuss about the (1) role of the stem cells in bone regeneration and their trophic factors and (2) the influence of stem cell microenvironment on the secretion of trophic factors and their effects on bone regeneration. The stem cell-based therapies using trophic factors may have profound clinical applications.

2. Stem cells and bone regeneration

Bone regenerates through complex and organized biological events of bone induction and conduction. This process involves a number of cell types and molecular signalling pathways in a defined sequence to maximize the repair and regeneration of the skeletal tissue. The organic matrix of the bone tissue is composed of collagen type I fibres (approximately 95%) proteoglycans and numerous non-collagenous proteins (5%) [7]. Non-collagenous proteins participate in the process of matrix maturation, mineralization and may regulate the functional activity of bone cells. Primarily, the functional integrity of bone tissue is maintained by the cell types such as osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells).

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During the phase of bone formation, the osteoblasts are recruited from mesenchymal stem cells (MSCs) present in bone marrow [8]. On the other hand, osteoclasts are derived from haematopoietic stem cells through committed osteoclast progenitors that fuse to form mature multinucleated cells [9]. The regeneration process occurs through osteogenesis initiated by skeletal stem cell also known as mesenchymal stem cells. During embryogenesis, the development of the skeletal tissue occurs by intramembranous and endochondral ossification. Bone formations begin with aggregation of MSCs to form condensations and within the mesenchymal condensation core; cells differentiate into chondrocytes in endochondral ossification or directly into osteoblasts in the intramembranous bone formation pathway [10]. Therefore, stem cells play a key role in bone regeneration and are considered to have the potential to treat bone defects either through cell-based therapies or tissue engineering. Stem cell-mediated bone regeneration provides a number of potential therapeutic advantages as compared to the use of autograft tissues. As such, therapeutic uses of stem cells are being explored extensively for bone tissue regeneration applications. MSCs and adipose-derived stem cells (ADSCs) have received considerable attention in this regard and have been extensively evaluated for bone regeneration.

Numerous studies in animal models clearly demonstrate that stem cells have the potential to treat critical-sized segmental defects, mandibular defects and effective spinal fusion to name a few. More recently, Liu et al. showed that systemic injection of MSCs into mandibular defects of dogs can increase new bone formation as compared to the defect without any cells [11]. Some clinical reports also suggest that MSCs and ADSCs can be used for treating fractures of the distal tibia, osteonecrosis of the femoral head and maxillary defects [12–16]. Although these results are promising, the efficacy of translating these outcomes into clinical practice at a large scale is still in infancy.

In addition to the cell-based therapies, stem cells are combined with biomaterials and implanted into the defect site and this tissue engineering approach is considered to be a promising strategy to treat bone defects. Numerous small animal studies have shown that treating the bone defects with a combination of biomaterials and MSCs can augment bone regeneration. Human bone marrow MSCs and macroporous calcium phosphate cement were combined and transplanted into critical-sized cranial defects in rats. The constructs generated much more new bone and blood vessels than the control calcium phosphate cement without cells [16]. Porous tantalum rods were implanted with or without autologous bone marrow stromal cells (BMSCs) on hind legs in dogs and the scaffold combined with cells enhanced new bone formation after 12 weeks of implantation [17]. These studies indicate that the combination of scaffolds with stem cells can enhance bone regeneration to greater extent. Likewise, composite scaffolds consisting of polycaprolactone and tricalcium phosphate (TCP) combined with autologous MSCs or recombinant human bone morphogenetic protein 7 was transplanted into critical-sized defects of the long bones of the sheep. The composite scaffold loaded with growth factor and MSCs was able to induce enhanced bone formation, indicating the importance of soluble factor in effective bone regeneration [18]. The osteogenic capability of ADSCs cells in healing critical-size mouse calvarial defects showed that implantation of apatite-coated poly lactic-co-glycolic acid scaffolds seeded with ADSCs can heal critical-size skel-

etal defects without genetic manipulation or the addition of exogenous growth factors [19, 20]. These animal studies with a combination of scaffolds and stem cells have shown great promise with excellent bone regeneration capabilities; however, translation of these into clinical use is limited. A study by Kawate et al. used a tissue engineering approach and transplanted β-TCP with MSCs and a free vascularized fibula into three young patients with steroid-induced osteonecrosis of the femoral head. Two out of the three patients showed healing of the defect with new bone formation and vascularization within 27 months of implantation. Although these studies with a tissue engineering approach was promising, problems still persist in terms of validating the source of the stem cells, the safety, the cost involved and more importantly understanding the molecular mechanisms involved and these questions has to be addressed before any clinical application can be achieved [21].

3. Stem cell trophic factors

In the recent past, stem cell technology has revolutionized the field of regenerative medicine and has been an attractive platform for the purposes of tissue repairs and cancer treatments. Stem cells are ideal for regenerative purposes due to their multipotentiality and self-renewing capabilities and this concept is fairly well established through many in vitro, in vivo and preclinical studies [22]. These cells play important roles in many biological processes, includ-

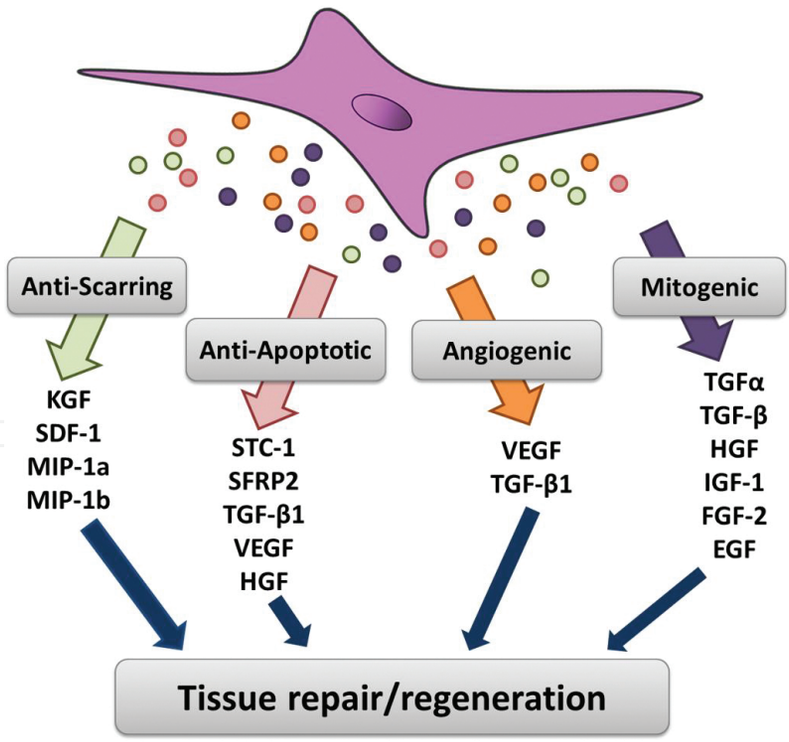

Figure 1. Various paracrine factors released by mesenchymal stem cells which play an important role in mitogenesis, angiogenesis, apoptosis and scarring. Endothelial Growth Factor (EGF), Fibroblast Growth Factor (FGF), Hepatocyte Growth Factor (HGF), Insulin Growth Factor (IGF), Kerotinocyte growth factor (KGF), Macrophage Inflammatory Proteins (MIP), Secreted Frizzled-Related Protein 2, Stanniocalcin-1(STC-1), Stromal cell derived factor (SDF), Transforming Growth Factor (TGF), Vascular Endothelial Growth Factor (VEGF).

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ing anti-inflammation, cell migration, proliferation and differentiation, and signal pathway activation or inhibition. There is strong understanding that stem cells (especially MSCs) are able to repair the tissue by modulating the environment they reside, influencing the immune response, supporting angiogenesis, and also through the productive cross talk with the resident cells as illustrated in schematic diagram (**Figure 1**). It is believed that the stem cells may be able to achieve these activities through the secretion of a broad panel of biomolecules called as trophic factors including growth factors, cytokines and chemokines and also the factors released in the extracellular vesicles (exosomes and microvesicles) [23–25]. Therefore, in recent years, researchers have a notion that the secret to the stem cell-based therapy may lie in the stem cell-secreted biomolecules rather than the cell itself as a therapeutic tool. Hence, there is a curious enthusiasm to explore and understand more about these secretion factors in order to enable a switch from use of stem cells to the use of stem cell secretion factors in regenerative medicine [22, 26]. MSCs are reported to secrete a variety of trophic factors such as transforming growth factors (TGF-α and β), hepatocyte growth factor (HGF), epithelial growth factor (EGF), basic fibroblast growth factor (FGF-2), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) which induces mitogenesis and angiogenesis and are also anti-apoptotic. Other trophic factors like interleukins, stromal cell-derived factor-1 and prostaglandin 2 are the key immunomodulatory cytokines [6, 25, 27, 28] as illustrated in **Figure 2**. Some fundamental studies have demonstrated that administration of stem cell condition medium containing bioactive factors released by the cells in culture medium can exert regenerative properties. Stem cell-derived secretory molecules has shown some promising tissue-repairing properties in cardiovascular [29], renal [6, 30], liver [31], lung injury [32], and neurodegenerative disease models [22, 33]. Trophic factors secreted by MSCs have induced proliferation of endogenous cardiac progenitor cells in vitro. Nakanishi et al. highlighted that conditioned media from rat MSCs can promote proliferation and migration of isolated cardiac progenitor cells and prevent their apoptosis when subjected to hypoxia and serum starvation [34]. Furthermore, human MSC secretions harvested in conditioned medium, reduced infarct size and preserved cardiac function in a large animal model of myocardial infarction [35]. MSC-conditioned media harvested after 24 h enhanced the paracrine effect and prevented oxygen-induced neonatal lung injury in a rat model [36]. An interesting study by Du et al. showed that MSC-conditioned media could even protect hepatocytes and sinusoidal endothelial cells and stimulate their regeneration in reduced-size rat liver transplantation [37]. The outcomes of this work was well complemented by another study undertaken by Van Poll et al., who provided clear evidence that introduction of MSC-conditioned media in a D-galactosamine-induced rat model of acute liver injury could enhance proliferation of hepatocytes and reduce apoptotic hepatocellular death, thereby increasing the survival rates and preventing hepatic failures [38]. The role of trophic factors in the treatment of chronic kidney disease is well demonstrated by Koppen et al. in a rat model. This study showed that administration of human embryonic MSC-conditioned media decreases the progression of chronic kidney disease with reduced hypertension and glomerular injury indicating the therapeutic benefits of trophic factors for kidney-related injuries and disease [39]. Most of the investigators have shown the beneficial effects of trophic factors from MSCs; however, it is not just confined to these cells alone. Adipose-derived stem cells which are also gaining popular-

ity as an attractive source of cells for regenerative purposes is showing similar properties. Sowa et al. showed that growth factors from ADSCs can promote peripheral nerve regeneration through paracrine secretion of trophic factors regardless of donor age or anatomic site of origin. The effects of mouse ADSCs-conditioned medium were tested on Schwann cells and dorsal root ganglion neurons in vitro. The results showed that ADSCs produced factors which were capable of promoting survival and proliferation of Schwann cells and enhancing the neurite outgrowth in dorsal root ganglion neurons in vitro [40]. Yamada et al. showed that ADSC-secreted molecules could induce a trophic effect in pancreatic islet culture conditions in vitro. These results suggested that trophic factors, particularly VEGF, secreted by human ADSCs enhanced the survival and function of porcine islet cells [41]. These studies suggest that the stem cell trophic factors alone have the potential for therapeutic use and can enhance effects in regeneration. The concept of utilizing the stem cell secretome for tissue repair is undoubtedly a step forward towards cell-free regenerative medicine and the effect of trophic factors in many other types of tissues like bone, ligament and for wound healing purposes is being explored.

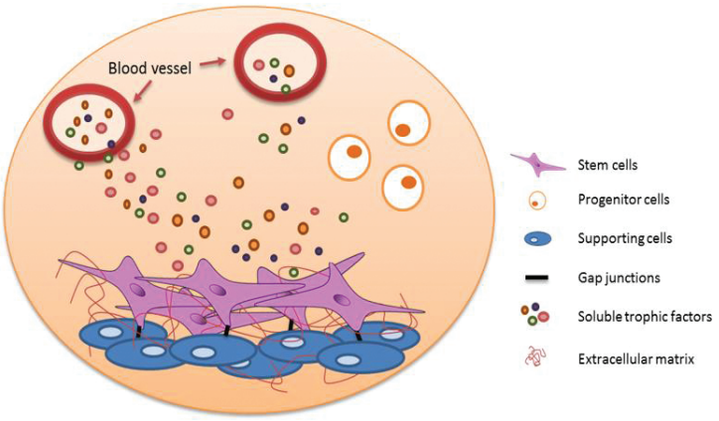

Figure 2. Architecture of the stem cell niche consisting of extracellular matrix, support cells, soluble trophic factors, transmembrane cell adhesion molecules and progenitor cells.

4. Stem cell trophic factors for bone regeneration

So far the use of stem cells in bone regeneration has largely been focussed on either transplanting the stem cells directly to the defect site or through the tissue engineering approaches. However, in recent years, the paracrine effects of stem cells in bone regeneration are being extensively explored and this can have positive implications in the field of bone regeneration. The secretion factors of the stem cells (e.g. MSCs) may have potential therapeutic applications in rheumatoid arthritis, osteoarthritis, genetic bone and cartilage disorders as well as bone metastasis [42]. The secretion factors released from the MSCs during their, osteogenic differentiation process induce recruitment and differentiation of endogenous progenitors. Murine MSCs was cultured in osteogenic medium and the condition media was

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collected and assessed for its effects on differentiation and migration of exogenous MSCs. The results showed that MSCs maintained in osteogenic medium, secreted factors at specific time points that induced alkaline phosphatase activity in exogenous MSCs as well as their migration thus showing the important contribution of trophic factors in the process of bone repair [43]. A study by Ando et al. showed that MSC-derived trophic factors can accelerate the healing process in distraction osteogenesis. In this study, serum-free conditioned medium derived from human MSCs was locally administered into the distraction gap in a high-speed distraction osteogenesis mouse model. The introduction of the MSC condition media supported the recruitment of murine bone marrow stromal cells and endothelial promoting osteoblast proliferation, differentiation and angiogenesis [44]. The role of trophic factors in treating rheumatoid arthritis is well demonstrated by Ishikawa et al. using dental pulp stem cells (DPSCs). This study showed that delivery of serum-free conditioned media from DPSCs into a collagen type II antibody-induced arthritis mouse model of the rheumatoid arthritis can inhibit inflammation-induced M2-type conditions I and suppress osteoclastogenesis and bone destruction in collagen type II antibody-induced arthritis [45]. An excellent study by Doorn et al. showed that trophic factors from human MSCs can contribute immensely to bone formation. In this study, human MSCs (hMSCs) were treated with small molecule dibutyryl cyclic adenosine monophosphate (db-cAMP) and the condition media was collected. This was used to culture a variety of cells including human umbilical vein endothelial cells, osteosarcoma, breast cancer and mouse myoblast cell line. The treatment of the condition media from cAMP-treated hMSCs to various cells could improve their proliferation and induce osteogenic differentiation with differential effects on migration. This study indicated that the trophic factors secreted by hMSCs can be tuned for a specific application [26, 46]. An in vitro study to investigate the role of brain-derived neurotrophic factor (BDNF) in spinal cord repair has shown that the condition media with the increased levels of BDNF was able to protect the motoneurons and enhance its survival rate, thus indicating the therapeutic benefits the trophic factors can have in the treatments of spinal cord injuries [47].

Furthermore, Cantinieaux et al. also showed the paracrine-mediated actions of bone marrow stromal cells for treating spinal cord injuries. An in vitro and in vivo study was conducted to evaluate the effects of factors released by the bone marrow stromal cells in a spinal cord injury in a rat model. The in vitro studies showed that bone marrow stromal cell-conditioned medium protected the neurons from apoptosis, activated macrophages and also exhibited some proangiogenic properties. Similar beneficial effects of trophic factors from the bone marrow stromal cells condition was also observed in the in vivo studies with histological analysis showing the proangiogenic action and tissue protection effect [48].

In addition, the effect of secretion factors from human umbilical cord-derived mesenchymal stem cells on the osteogenesis of human MSCs has shown to initiate osteogenic differentiation with increased amount of calcium deposit, and upregulation of osteogenic gene expression [49]. These outcomes suggests that stem cell trophic factors may have key solutions for bone repair and regenerations and further mechanistic understanding can reveal their potential for further clinical applications.

5. Stem cell microenvironment: biophysical cues and trophic factors

Stem cells reside in complicated and dynamic three-dimensional (3D) microenvironments in vivo known as stem cell niches where they undergo self-renewal and differentiation. Their function is maintained through an array of complex signals derived from this niche [50–52]. Structurally, the niche is composed of extracellular matrix (ECM), soluble proteins like chemokines, cytokines and growth factors, supporting cells and physical factors. [53, 54]. ECM proteins are the influential components of the niche and they primarily help in maintaining stem cell homoeostasis and direct lineage commitment. ECM forms the vital communication network for transferring the cell signals emanated from soluble and matrix-bound factors and from cell-matrix interactions and the composition of the ECM can govern the fate of the cells considerably [55–57]. This concept is very well demonstrated through the preservation of the decellularized matrix which was able to guide stem cell differentiation into the cell types residing in the tissue from which the ECM was derived. On the basis of these properties, decellularized organs have been used in tissue engineering and for developing cell therapy approaches [58, 59]. ECM parameters are extremely dynamic and are spatially and temporarily controlled during development suggesting that they play a morphogenetic role in guiding differentiation and arrangement of cells. Support cells play an important role in restricting the stem cells to their niche through the cell surface adhesion proteins. The interactions between the stem cells and the support cells are largely governed by cadherin proteins that form adherens junctions. As such, stem cells are able to maintain their stemness and self-renewal when they are in the vicinity of the support cells [60]. Once the cell divides, one daughter cell remains in contact with the support cell and the other migrates from the niche and commits itself to a particular lineage [61]. Recent study by Polisetti et al. provided excellent insights into molecular mechanisms of progenitor cell niche anchorage mediated by integrins-, cadherins- and dystrophin-associated proteins that regulates both stable and dynamic cell-matrix and cell-cell interactions within the limbal niche [62]. Additionally, support cells such as perivascular stromal cells including nestin+ mesenchymal stromal cell (MSC) [63], leptin receptor (Lepr) expressing mesenchymal cell and Mx1+ stromal cells [64] have shown to play vital roles in regulating the functions of hematopoietic cells in bone marrow. Support cells such as osteoblasts were believed to play an important role in preserving the HSCs in a quiescent state and help in their maintenance or just form a niche supportive of early lymphoid progenitors [65, 66]. These studies suggest the importance of support cells in the architecture of stem cell niche which eventually affects the functions of stem cells [67]. Soluble molecules are another important component of the niche besides ECM and support cells and are absolutely critical for directing the stem cell fate. Soluble factors can be in the form of growth factors, cytokines, enzymes, transforming growth factors, bone morphogenic factors and vitamin C to name a few [68, 69]. These factors can be either added to the culture conditions or secreted by the stem cells or the supporting cells in the niche. These factors can then bind to the membrane receptors of the cells and trigger the cell signalling pathways altering the gene expression of the stem cells [61, 70, 71]. Soluble molecules that are prevalent in most of the niches include Wnts, hedgehog proteins, FGF and bone morphogenetic proteins (BMP) . Some of these molecules are key factors in regulating the self-renewal of haematopoietic stem

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cells. It is well established through many studies that Wnts and hedgehog proteins play a key role in osteogenesis especially in bone formation, maintaining cellular differentiation and regulating the formation of bone and cartilage, whereas FGFs and BMPs have profound impact on the osteoprogenitor differentiation and the regulation of the endochondral and intramembranous ossification [60, 72]. Similarly, soluble growth factors and cell membrane-bound factors such as platelet-derived growth factor (PDGF), TGF-β, VEGF, BMP and Notch signalling have been implicated as having multivariable effects on cardiac development [73, 74]. In recent years, it has been established that the stem cell niche is not just confined to the soluble factors and cell-cell interactions but also to the definable physical and mechanical cues, which influences the decision-making capability of the stem cells. A number of investigators have demonstrated that stem cells have the ability to sense and transduce the physical and mechanical cues. The cues such as mechanical strain, stiffness, shear stress and topography can regulate the fate of the cells. It has been shown that application of mechanical strain can increase proliferation and decrease differentiation in human embryonic cells, which affects the cell alignment in the direction of the strain and also directs the MSCs to myogenic phenotype. It has been shown that MSCs can express high levels of ligament-specific markers under the influence of rotational strain [75–78]. Additionally, the fate of the stem cells can also be influenced by the substrate stiffness. The effect of stiffness on stem cell differentiation has been well demonstrated by numerous investigators and an excellent study by Engler et al. showed that variation in the substrate stiffness can modulate the differentiation of stem cells into various specific lineages such as neuronal, muscle and osteogenic lineages. This lineage commitment was found to depend largely on the elasticity of the substrate [53]. A similar result was highlighted by Saha et al., and Banerjee et al., who showed that changes in the substrate stiffness can present a defining influence on the differentiation of neural progenitor cells with stiff substrates modulating the cells to astrocytes while the softer ones towards neuronal differentiation [79, 80]. Stem cells are also sensitive to the shear stress and this can result in the morphological changes which in turn affects the cell behaviour at the molecular level. A study by Illi et al. showed that shear stress can stimulate the molecular pathways leading to histone modifications in mouse embryonic stem cells resulting in epigenomic regulations [81]. In addition, the shear stress has been shown to affect the differentiation of stem cells into vascular cells. Exposure of stem cells to shear stress increased the expression of endothelial cell-specific markers such as von Willebrand factor and vascular endothelial-cadherin [75]. Topographical cues can also influence various behaviours of the stem cells similar to stress, strain and stiffness. Topographical modifications in the substrate can change the morphological features of the cells which can vary the orientation of the cytoskeletal structure of the cells thus affecting the function of the stem cell fate. Dalby et al. showed the influence of ordered and disordered nanoscale pattern on differentiation of MSCs. This study showed that osteogenesis was more predominant with ordered nanoscale pattern with an increase in the expression of genes responsible for osteogenic differentiation as compared to the disordered pattern [82]. A similar outcome was reported by Zouani et al. who showed that substrates with large nanodepths (100 μm) induced higher osteogenic differentiation as compared to the small depths (10 μm) indicating that stem cells can sense and respond to the topographical changes and regulate their function [83–85].

Such studies have convinced that the mechanical cues can influence the fate of the stem cells; however, the mechanistic insights as to how these cues direct the differentiation of stem cells is just beginning to be unravelled. Stem cells can sense the stiff microenvironment and transduce the signals through the Rho kinase [86, 87], TGF-β [88, 89], Src family kinases [90] and phosphor-tyrosine signalling pathways [91, 92]. Other studies by Dupont et al. and Swift et al. have shown that yes-associated protein and transcriptional coactivator with PDZ-binding motif also have a significant role in regulating the stem cell differentiation mechanism in response to mechanical parameters. While more and more mechanistic data begins to emerge, it is only clear that mechanical cues are potent physical parameters in the regulation of stem cell differentiation [93–96]. The release and beneficial effects of trophic factors from the stem cells will also depend on the microenvironment it is residing in. Changes in the microenvironment with respect to the biological and mechanical cues can affect the release of trophic factors which can have profound implications in their functionality. More studies are now beginning to emerge to decipher these concepts using various artificial platforms. For example, Abdeen et al. studied the combined role of stiffness and matrix protein on the secretory profile of MSCs and their effects on human microvascular endothelial cells. In this work, the conditioned media from MSCs adherent to polyacrylamide hydrogel with controlled matrix rigidity and protein composition was collected and applied to a model angiogenesis assay using HMVECs within Matrigel. The result from this study showed that secretion of the trophic factors was related to a combined effect of stiffness and adhesion protein for directing proangiogenic signalling [97]. Jose et al. pretreated the MSCs with glycine-histidine-lysine (GHK), a peptide fragment of osteonectin and a matrix cellular protein with reported proangiogenic potential. The study revealed a dose-dependent increase in VEGF concentration in media conditioned by GHK-treated MSC, which increased endothelial cell proliferation, migration and tubule formation. This study suggested that microenvironment of the stem cells can have significant influence on the trophic factors and their functionality [98]. Furthermore, Silva et al. showed that the secretome of the bone marrow MSCs were affected when the cells were cultured on fibronectin peptide-modified hydrogels as compared to the unmodified gels and this change in the secretome-induced higher metabolic viabilities and neuronal cell densities [99]. Hoch et al. also showed that cell-secreted decellularized extracellular matrices can preserve the bone-forming phenotype of the differentiated MSC. In this study, osteogenically induced MSCs were cultured on the decellularized matrices and the osteogenic and angiogenic potential was measured after the withdrawal of the induction media. It was found that culturing osteogenically induced MSCs on decellularized matrix can enhance calcium deposition and secretion of proangiogenic factor such as VEGF [100].

It has also been noted that the changes in the microenvironment of the stem cells due to biomolecules can also affect the trophic factors secretion by the stem cells and this can in turn affect the functionality of the cells by our group and others. We have shown that short-term exposure of human osteoblasts to tumour necrosis factor (TNF-α) can promote osteogenic differentiation and also stimulate human osteoblasts to secrete soluble factors that can foster a microenvironment favouring osteogenic differentiation of ADSC [101]. A similar study was performed by Czekanska et al., whereby MSCs were stimulated with interleukin-1β (IL1β), granulocyte-colony stimulating factor (GCSF), stromal cell-derived factor 1 (SDF1) and stem

cell factor (SCF) for about 2 h. The results showed that a mere 2-h stimulation could affect the expression of multiple cytokine genes and proteins in MSC significantly. IL1β strongly promoted the secretion of a wide range of proteins with chemotactic, proinflammatory and angiogenic properties, whereas SCF regulated the expression of proteins involved in proliferation, chondrogenesis and ECM regulation. This outcome was clear evidence that the changes in secretome can be directed towards a desired final functional outcome through the selection of the most appropriate cytokine [102, 103].

Through numerous studies reported in the literature, it is quite evident that the stem cell function greatly depends on the architecture of the niche; any physical or biochemical disruption can affect the stem cell function profoundly.

6. Conclusions

Stem cells are in a way considered to be the "building blocks" of regenerative medicine and are believed to possess solutions to many types of injuries and diseases. Enormous amount of research is focussed on deciphering and understanding the functionalities of these cells through the cell-based therapy, through tissue engineering or purely through their paracrine activities.

The idea of "stem cell free regenerative" medicine has undoubtedly captured a great deal of attention in the recent few years. Most studies suggest that the use of stem cells secretory molecules such as trophic factors, microvesicles or exosomes can be advantageous and valuable in the treatment of injuries or diseases compared to the cell-based therapies. There are more to be explored in terms of their mechanisms at a molecular level, the effect of microenvironment on their release, and the long-term effects of these kinds of treatments in an in vivo scenario. Answers to these questions can help in validating cell-free regenerative technology as a potential therapeutic tool.

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References

[1] Australian Orthopedic Association, 2014. https://www.aoa.org.au/ accessed on 12/11/15

[2] Amini, A.R., C.T. Laurencin, and S.P. Nukavarapu, *Bone tissue engineering: recent advances and challenges*. Critical Reviews in Biomedical Engineering, 2012. 40(5): p. 363–408.

[3] Marino, J.T. and B.H. Ziran, *Use of solid and cancellous autologous bone graft for fractures and nonunions*. Orthopedic Clinics of North America, 2010. 41(1): p. 15–26.

[4] Dimitriou, R., et al., *Bone: regeneration current concepts and future directions*. BioMed Central, 2011. 9(66): p. 1–28.

[5] Walmsley, G., et al., *Osteogenic differentiation of adipose-derived stromal cells: advancements and future directions for bone tissue engineering*. Science Proceedings, 2016. 3: p. 1–6.

[6] Tsuji, K. and S. Kitamura, *Trophic factors from tissue stem cells for renal regeneration*. Stem Cells International, 2015. 2015: p. 7.

[7] Barrère, F., C.A. van Blitterswijk, and K. de Groot, *Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics*. International Journal of Nanomedicine, 2006. 1(3): p. 317–332.

[8] Logar, D., et al., *Expression of bone resorption genes in osteoarthritis and in osteoporosis*. Journal of Bone and Mineral Metabolism, 2007. 24(4): p. 219–225.

[9] Nakamura, I., et al., *Involvement of αvβ3 integrins in osteoclast function*. Journal of Bone and Mineral Metabolism, 2007. 25(6): p. 337–344.

[10] Deschaseaux, F., L. Sensébé, and D. Heymann, *Mechanisms of bone repair and regeneration*. Trends in Molecular Medicine, 2009. 15(9): p. 417–429.

[11] Liu, X., et al., *Mesenchymal stem cells systemically injected into femoral marrow of dogs home to mandibular defects to enhance new bone formation*. Tissue Engineering Part A, 2013. 20(3–4): p. 883–892.

[12] Liebergall, M., et al., *Stem cell-based therapy for prevention of delayed fracture union: a randomized and prospective preliminary study*. Molecular Therapy, 2013. 21(8): p. 1631–1638.

[13] Mesimäki, K., et al., *Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells*. International Journal of Oral and Maxillofacial Surgery, 2009. 38(3): p. 201–209.

[14] Gangji, V., V. De Maertelaer, and J.-P. Hauzeur, *Autologous bone marrow cell implantation in the treatment of non-traumatic osteonecrosis of the femoral head: Five year follow-up of a prospective controlled study*. Bone, 2011. 49(5): p. 1005–1009.

[15] Zhao, D., et al., *Treatment of early stage osteonecrosis of the femoral head with autologous implantation of bone marrow-derived and cultured mesenchymal stem cells*. Bone, 2012. 50(1): p. 325–330.

[16] Chen, W., et al., *Umbilical cord and bone marrow mesenchymal stem cell seeding on macroporous calcium phosphate for bone regeneration in rat cranial defects*. Biomaterials, 2013. 34(38): p. 9917–9925.

[17] Wei, X., et al., *Tantalum coating of porous carbon scaffold supplemented with autologous bone marrow stromal stem cells for bone regeneration in vitro and in vivo*. Experimental Biology and Medicine, 2016. 241: p. 592–602.

[18] Reichert, J.C., et al., *A Tissue Engineering Solution for segmental defect regeneration in load-bearing long bones*. Science Translational Medicine, 2012. 4(141): p. 141ra93.

[19] Cowan, C.M., et al., *Adipose-derived adult stromal cells heal critical-size mouse calvarial defects*. Nature Biotechnology, 2004. 22(5): p. 560–567.

[20] Levi, B., et al., *Human adipose derived stromal cells heal critical size mouse calvarial defects*. PLoS One, 2010. 5(6): p. e11177.

[21] Kawate, K., et al., *Tissue-engineered approach for the treatment of steroid-induced osteonecrosis of the femoral head: transplantation of autologous mesenchymal stem cells cultured with beta-tricalcium phosphate ceramics and free vascularized fibula*. Artificial Organs, 2006. 30(12): p. 960–962.

[22] Bollini, S., et al., *The regenerative role of the fetal and adult stem cell secretome*. Journal of Clinical Medicine, 2013. 2(4): p. 302–327.

[23] Baglio, S.R., D.M. Pegtel, and N. Baldini, *Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy*. Frontiers in Physiology, 2012. 3(359.10): p. 3389.

[24] Gallina, C., V. Turinetto, and C. Giachino, *A new paradigm in cardiac regeneration: the mesenchymal stem cell secretome*. Stem Cells International, 2015. 2015(765846): p. 765846.

[25] Lavoie, J.R. and M. Rosu-Myles, *Uncovering the secretes of mesenchymal stem cells*. Biochimie, 2013. 95(12): p. 2212–2221.

[26] Doorn, J., et al., *Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements*. Tissue Engineering Part B: Reviews, 2011. 18(2): p. 101–115.

[27] Maumus, M., C. Jorgensen, and D. Noël, *Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes*. Biochimie, 2013. 95(12): p. 2229–2234.

[28] Murphy, M.B., K. Moncivais, and A.I. Caplan, *Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine*. Experimental & Molecular Medicine, 2013. 45(11): p. e54.

[29] Mirotsou, M., et al., *Paracrine mechanisms of stem cell reparative and regenerative actions in the heart*. Journal of Molecular and Cellular Cardiology, 2011. 50(2): p. 280–289.

[30] Cantaluppi, V., et al., *Rationale of mesenchymal stem cell therapy in kidney injury*. American Journal of Kidney Diseases, 2013. 61(2): p. 300–309.

[31] Kuo, T.K., et al., *Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells*. Gastroenterology, 2008. 134(7): p. 2111–2121. e3.

[32] Gotts, J.E. and M.A. Matthay, *Mesenchymal stem cells and acute lung injury*. Critical Care Clinics, 2011. 27(3): p. 719–733.

[33] Uccelli, A., et al., *Neuroprotective features of mesenchymal stem cells*. Best Practice & Research Clinical Haematology, 2011. 24(1): p. 59–64.

[34] Nakanishi, C., et al., *Activation of cardiac progenitor cells through paracrine effects of mesenchymal stem cells*. Biochemical and Biophysical Research Communications, 2008. 374(1): p. 11–16.

[35] Timmers, L., et al., *Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction*. Stem Cell Research, 2011. 6(3): p. 206–214.

[36] Waszak, P., et al., *Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats*. Stem Cells and Development, 2012. 21(15): p. 2789–2797.

[37] Du, Z., et al., *Mesenchymal stem cell–conditioned medium reduces liver injury and enhances regeneration in reduced-size rat liver transplantation*. Journal of Surgical Research, 2013. 183(2): p. 907–915.

[38] van Poll, D., et al., *Mesenchymal stem cell–derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo*. Hepatology, 2008. 47(5): p. 1634–1643.

[39] van Koppen, A., et al., *Human embryonic mesenchymal stem cell-derived conditioned medium rescues kidney function in rats with established chronic kidney disease*. PLoS One, 2012. 7(6): p. e38746.

[40] Sowa, Y., et al., *Adipose-derived stem cells produce factors enhancing peripheral nerve regeneration: influence of age and anatomic site of origin*. Stem Cells and Development, 2011. 21(11): p. 1852–1862.

[41] Yamada, S., et al., *Trophic effect of adipose tissue–derived stem cells on porcine islet cells*. Journal of Surgical Research, 2014. 187(2): p. 667–672.

[42] Djouad, F., et al., *Mesenchymal stem cells: innovative therapeutic tools for rheumatic diseases*. Nature Reviews Rheumatology, 2009. 5(7): p. 392–399.

[43] Li, F., N. Whyte, and C. Niyibizi, *Differentiating multipotent mesenchymal stromal cells generate factors that exert paracrine activities on exogenous MSCs: Implications for paracrine activities in bone regeneration*. Biochemical and Biophysical Research Communications, 2012. 426(4): p. 475–479.

[44] Ando, Y., et al., *Stem cell-conditioned medium accelerates distraction osteogenesis through multiple regenerative mechanisms*. Bone, 2014. 61: p. 82–90.

[45] Ishikawa, J., et al., *Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis*. Bone, 2016. 83: p. 210–219.

[46] Doorn, J., et al., *Pro-osteogenic trophic effects by PKA activation in human mesenchymal stromal cells*. Biomaterials, 2011. 32(26): p. 6089–6098.

[47] Ritfeld, G.J., et al., *The role of brain-derived neurotrophic factor in bone marrow stromal cell-mediated spinal cord repair*. Cell Transplantation, 2015. 24(11): p. 2209–2220.

[48] Cantinieaux, D., et al., *Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation*. PLoS One, 2013. 8(8): p. e69515.

[49] Wang, K.-X., et al., *The effects of secretion factors from umbilical cord derived mesenchymal stem cells on osteogenic differentiation of mesenchymal stem cells*. PLoS One, 2015. 10(3): p. e0120593.

[50] Thomson, J.A., et al., *Embryonic stem cell lines derived from human blastocysts*. Science, 1998. 282(5391): p. 1145–1147.

[51] Takahashi, K. and S. Yamanaka, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors*. Cell, 2006. 126(4): p. 663–676.

[52] Watt, F.M. and B.L. M. Hogan, *Out of Eden: Stem cells and their niches*. Science, 2000. 287(5457): p. 1427–1430.

[53] Engler, A.J., et al., *Matrix elasticity directs stem cell lineage specification*. Cell, 2006. 126(4): p. 677–689.

[54] Ward Jr., D.F., et al., *Mechanical strain enhances extracellular matrix-induced gene focusing and promotes osteogenic differentiation of human mesenchymal stem cells through an extracellular-related kinase-dependent pathway*. Stem Cells and Development, 2007. 16(3): p. 467–480.

[55] Brizzi, M.F., G. Tarone, and P. Defilippi, *Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche*. Current Opinion in Cell Biology, 2012. 24(5): p. 645–651.

[56] Peerani, R. and P.W. Zandstra, *Enabling stem cell therapies through synthetic stem cell–niche engineering*. The Journal of Clinical Investigation, 2010. 120(1): p. 60–70.

[57] Discher, D.E., D.J. Mooney, and P.W. Zandstra, *Growth factors, matrices, and forces combine and control stem cells*. Science, 2009. 324(5935): p. 1673–1677.

[58] Gattazzo, F., A. Urciuolo, and P. Bonaldo, *Extracellular matrix: a dynamic microenvironment for stem cell niche*. Biochimica et Biophysica Acta (BBA)-General Subjects, 2014. 1840(8): p. 2506–2519.

[59] Nakayama, K.H., et al., *Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering*. Tissue Engineering Part A, 2010. 16(7): p. 2207–2216.

[60] Lutolf, M.P. and H.M. Blau, *Artificial stem cell niches*. Advanced Materials, 2009. 21(32–33): p. 3255–3268.

[61] Nava , M.M., M.T. Raimondi, and R. Pietrabissa, *Controlling self-renewal and differentiation of stem cells via mechanical cues*. BioMedical Research International, 2012. 2012 p. 797410.

[62] Polisetti, N., et al., *Cell adhesion molecules and stem cell-niche-interactions in the limbal stem cell niche*. Stem Cells, 2016. 34(1): p. 203–219.

[63] Méndez-Ferrer, S., et al., *Mesenchymal and haematopoietic stem cells form a unique bone marrow niche*. Nature, 2010. 466(7308): p. 829–834.

[64] Park, D., et al., *Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration*. Cell Stem Cell, 2012. 10(3): p. 259–272.

[65] Calvi, L., et al., *Osteoblastic cells regulate the haematopoietic stem cell niche*. Nature, 2003. 425(6960): p. 841–846.

[66] Zhang, J., et al., *Identification of the haematopoietic stem cell niche and control of the niche size*. Nature, 2003. 425(6960): p. 836–841.

[67] Kumar, B., et al., *Exosome-mediated microenvironment dysregulation in leukemia*. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2016. 1863(3): p. 464–470.

[68] Kawaguchi, J., P.J. Mee, and A.G. Smith, *Osteogenic and chondrogenic differentiation of embryonic stem cells in response to specific growth factors*. Bone, 2005. 36(5): p. 758–769.

[69] Zhang, H., et al., *Biomimetic three-dimensional microenvironment for controlling stem cell fate*. Interface Focus, 2011. 1: p. rsfs20110035.

[70] Reilly, G.C. and A.J. Engler, *Intrinsic extracellular matrix properties regulate stem cell differentiation*. Journal of Biomechanics, 2010. 43(1): p. 55–62.

[71] Liu, Z.J., Y. Zhuge, and O.C. Velazquez, *Trafficking and differentiation of mesenchymal stem cells*. Journal of Cellular Biochemistry, 2009. 106(6): p. 984–991.

[72] Arvidson, K., et al., *Bone regeneration and stem cells*. Journal of Cellular and Molecular Medicine, 2011. 15(4): p. 718–746.

[73] Arshi, A., et al., *Rigid microenvironments promote cardiac differentiation of mouse and human embryonic stem cells*. Science and Technology of Advanced Materials, 2016.

[74] Yamashita, J., et al., *Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors*. Nature, 2000. 408(6808): p. 92–96.

[75] Shitiz, K., et al., *Control of stem cell fate and function by engineering physical microenvironments*. Integrative Biology, 2012. 4(9): p. 1008–1018.

[76] Altman, G., et al., *Cell differentiation by mechanical stress*. Faseb Journal, 2002. 16(2): p. 270–272.

[77] Saha, S., et al., *Inhibition of human embryonic stem cell differentiation by mechanical strain*. Journal of cellular physiology, 2006. 206(1): p. 126–137.

[78] Park, J.S., et al., *Differential effects of equiaxial and uniaxial strain on mesenchymal stem cells*. Biotechnology and Bioengineering, 2004. 88(3): p. 359–368.

[79] Saha, K., et al., *Substrate modulus directs neural stem cell behavior*. Biophysical Journal, 2008. 95(9): p. 4426–4438.

[80] Banerjee, A., et al., *The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells*. Biomaterials, 2009. 30(27): p. 4695–4699.

[81] Illi, B., et al., *Epigenetic histone modification and cardiovascular lineage programming in mouse embryonic stem cells exposed to laminar shear stress*. Circulation Research, 2005. 96(5): p. 501–508.

[82] Dalby, M.J., et al., *The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder*. Nature Materials, 2007. 6(12): p. 997–1003.

[83] Griffin, M.F., et al., *Control of stem cell fate by engineering their micro and nanoenvironment*. World Journal of Stem Cells, 2015. 7(1): p. 37.

[84] Viswanathan, P., et al., *3D surface topology guides stem cell adhesion and differentiation*. Biomaterials, 2015. 52: p. 140–147.

[85] Zouani, O.F., et al., *Altered nanofeature size dictates stem cell differentiation*. Journal of Cell Science, 2012. 125(5): p. 1217–1224.

[86] Provenzano, P.P. and P.J. Keely, *Mechanical signaling through the cytoskeleton regulates cell proliferation by coordinated focal adhesion and Rho GTPase signaling*. Journal of Cell Science, 2011. 124(8): p. 1195–1205.

[87] Chaudhuri, P.K., et al., *Topography induces differential sensitivity on cancer cell proliferation via Rho-ROCK-Myosin contractility*. Scientific Reports, 2016. 6: p. 19672.

[88] Allen, J.L., M.E. Cooke, and T. Alliston, *ECM stiffness primes the TGFβ pathway to promote chondrocyte differentiation*. Molecular Biology of The Cell, 2012. 23(18): p. 3731–3742.

[89] Horiguchi, M., M. Ota, and D.B. Rifkin, *Matrix control of transforming growth factor-β function*. Journal of Biochemistry, 2012. 152(4): p. 321–329.

[90] Jasaitis, A., et al., *E-Cadherin-dependent stimulation of traction force at focal adhesions via the Src and PI3K signaling pathways*. Biophysical Journal, 2012. 103(2): p. 175–184.

[91] Arnsdorf, E.J., et al., *Mechanically induced osteogenic differentiation–the role of RhoA, ROCKII and cytoskeletal dynamics*. Journal of Cell Science, 2009. 122(4): p. 546–553.

[92] Sun, Y., C.S. Chen, and J. Fu, *Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment*. Annual Review of Biophysics, 2012. 41: p. 519.

[93] Shin, J.-W. and D.J. Mooney, *Improving stem cell therapeutics with mechanobiology*. Cell Stem Cell, 2016. 18(1): p. 16–19.

[94] Ivanovska, I.L., et al., *Stem cell mechanobiology: diverse lessons from bone marrow*. Trends in Cell Biology, 2015. 25(9): p. 523–532.

[95] Dupont, S., et al., *Role of YAP/TAZ in mechanotransduction*. Nature, 2011. 474(7350): p. 179–183.

[96] Halder, G., S. Dupont, and S. Piccolo, *Transduction of mechanical and cytoskeletal cues by YAP and TAZ*. Nature Reviews Molecular Cell Biology, 2012. 13(9): p. 591–600.

[97] Abdeen, A.A., et al., *Matrix composition and mechanics direct proangiogenic signaling from mesenchymal stem cells*. Tissue Engineering Part A, 2014. 20(19–20): p. 2737–2745.

[98] Jose, S., et al., *Enhanced trophic factor secretion by mesenchymal stem/stromal cells with glycine-histidine-lysine (GHK)-modified alginate hydrogels*. Acta Biomaterialia, 2014. 10(5): p. 1955–1964.

[99] Silva, N.A., et al., *Modulation of bone marrow mesenchymal stem cell secretome by ECM-like hydrogels*. Biochimie, 2013. 95(12): p. 2314–2319.

[100] Hoch, A.I., et al., *Cell-secreted matrices perpetuate the bone-forming phenotype of differentiated mesenchymal stem cells*. Biomaterials, 2016. 74: p. 178–187.

[101] Lu, Z., et al., *Short-term exposure to tumor necrosis factor-alpha enables human osteoblasts to direct adipose tissue-derived mesenchymal stem cells into osteogenic differentiation*. Stem Cells and Development, 2012. 21(13): p. 2420–2429.

[102] Czekanska, E.M., et al., *Enhancing inflammatory and chemotactic signals to regulate bone regeneration*. European Cells & Materials, 2014. 28: p. 320–334.

[103] Crowder, S.W., et al., *Material cues as potent regulators of epigenetics and stem cell function*. Cell Stem Cell, 2016. 18(1): p. 39–52.